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Possible Role of the Nectar-Guide-like Mark in Flower Explosion in *Desmodium paniculatum* (L.) DC. (Leguminosae)

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Desmodium paniculatum, which is from North America and is naturalized widely in Japan, exhibits characteristics typical to explosive flowers, i. e., has no return to original position in the wings and keel, spreads a pollen cloud at flower explosion, rarely has re-visits by pollinators, and is nectarless. The explosion is induced by bee-proboscis insertion into the opening between the standard- and the wing-base, with no other force needed. The flower possesses marked spots in the basal part of the standard, that are very similar to the nectar guides common in the nectariferous flowers of Papilionoideae. However, they are not guide marks to introduce bees to reward objects, because it does not have any reward in the basal part. They appear to function as a guide mark to help bees make the flower explode. Bees can obtain the pollen reward only after insertion of their proboscis into the opening under the mark.

Key words: *Desmodium paniculatum*, explosive flower, Leguminosae, nectar guide, pollen guide.

Flowers that provide their pollinators nectar as a reward, especially those with hidden nectaries inside, usually possess nectar guides (Sprengel 1793). Pollinators can easily approach the nectaries by their help. In Papilionoideae of the family Leguminosae, most nectariferous flowers have nectar guides in the basal part of the standard. Their nectary is at the base of the ovary and insects can take nectar through holes in the base of the filaments with their proboscis (Faegri and van der Pijl 1971). The nectary is invisible because it is covered with the basal parts of petals. Bees, the most common pollinators, land on the wing-keel complex and insert their proboscis into the opening under the nectar guide. The opening, which is between the standard- and the wing-base, introduces the proboscis of nectar-feeding insects into the basal holes of the filament-tube, and was referred to as a tongue-guide by Westerkamp (1997). During this process the anthers and the stigma in the wing-keel complex come out to touch the bee abdomen. The pollen grains dusting the abdomen may be a secondary reward for the pollinators.

Legume species with typical explosive flowers present a pollination mechanism a little different from most species with non-explosive flowers. The stamens and pistil in the flowers of the genera such as *Medicago*, *Genista* and *Cytisus* are rapidly released from the wing-keel complex by a visit of their pollinators, and never come back into the keel. After explosion the flowers are seldom visited again by the pollinators (Kugler 1955, Faegri and van der Pijl 1971, Proctor and Yeo 1973, Lopez et al. 1999). Pollen is almost the only reward for the pollinators in these flowers (Kugler 1955, Faegri and van

der Pijl 1971), although *Mdeicago* spp. secrete much nectar (Free 1993, Perez-Banon et al. 2003) and Galloni and Cristofolini (2003) detected glucose at the base of the standard in *Cystisophyllum* and *Spartium* and at the filament-tube in *Genista*. Westerkamp (1997) pointed out that the tongue-guide existed even in the papilionate flowers that did not provide nectar, and that bees were constrained into a position suitable for effecting pollination. However, there are few studies addressing how the pollinators know where the anthers, which are hidden in the keel-wing complex, are located and how they obtain pollen in the flowers providing pollen as the main reward.

Many species of the genus *Desmodium* have explosive flowers (Faegri and van der Pijl 1971, Ohashi et al. 1981), and their explosion is caused by some additional force exerted from outside by the pollinator (Faegri and van der Pijl 1971). In *Desmodium*, however, there are no reports on what is the most important force to cause explosion. The flowers of *Desmodium paniculatum* (L.) DC, a species from North America and naturalized widely in Japan, are exploded by a bee-visit. They have marked spots like a nectar guide in the basal area of the standard, although no nectar appears to be produced. I will show the elegant explosion of a *D. paniculatum* flower by insertion of bee proboscis into the opening, and discuss the possible role of the standard spots in the explosion.

Materials and Methods

Field investigations were made in the plant populations of Yanagido, Sano and Hinakura in Gifu City.

Anthers were counted in the flowers that had just had a bee visitation and in ones that were artificially explored with a needle.

UV absorption of the corolla was examined through photographs taken by a digital camera (Canon EOS 10D with a Canon Macro EF 100 mm lens and a Kenko UV 360 filter) under artificial UV light. The color photographs were changed to monochrome with a computer using Adobe Photoshop 6.0. These monochrome photographs are quite similar to ones made from monochrome-film (Takahashi unpublished).

Glucose reaction was examined in various parts of the flower with glucose-test paper (UROPIECE S made by Toyo Roshi Kaisha Ltd.) to check nectar exudation. The test paper was cut into small stripes (about 1 mm in width) if needed. The paper was softly pressed against each testable part of the flower to avoid injuring the tissues.

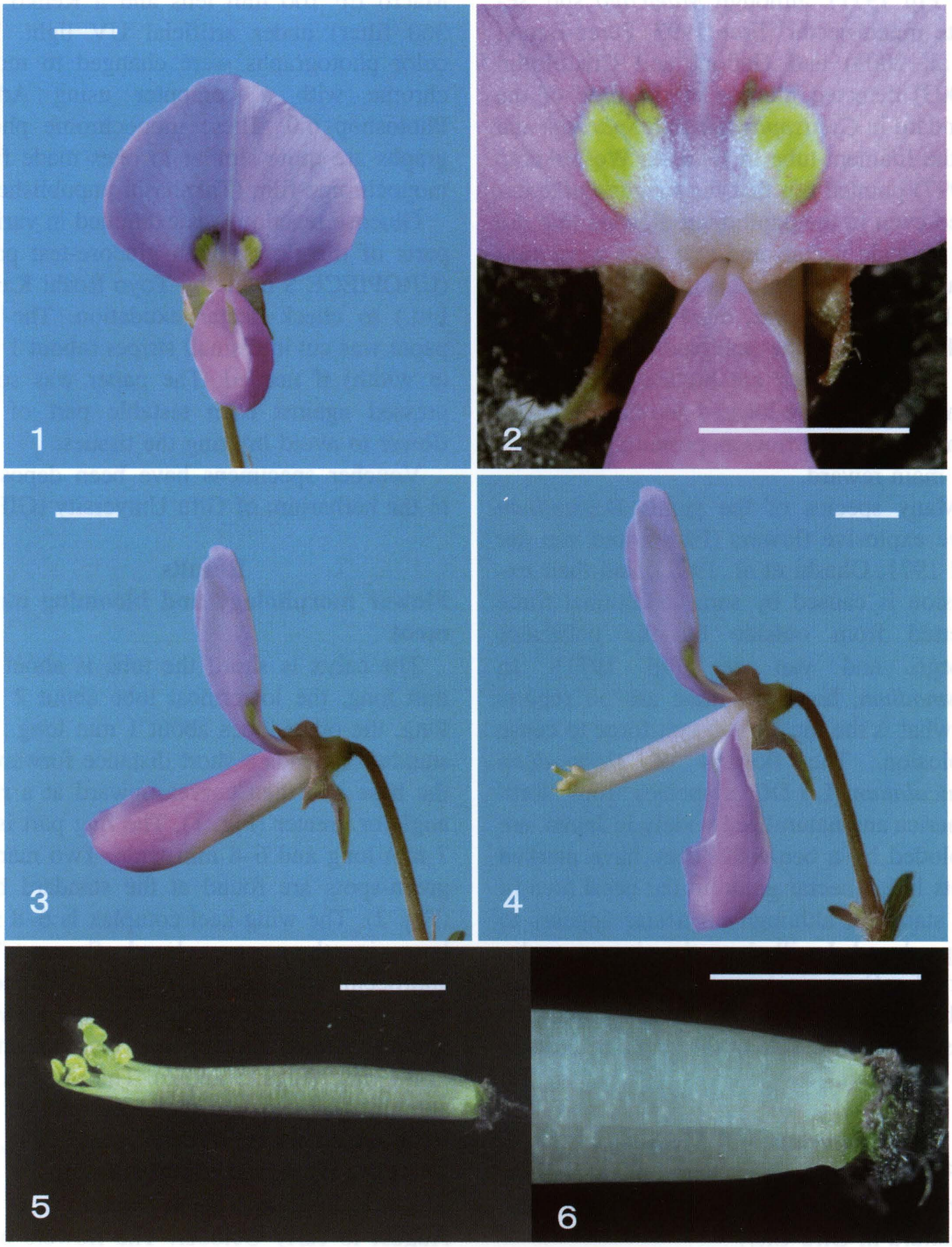
Voucher specimens have been deposited in the herbarium of Gifu University (GIFU).

Results

Flower morphology and blooming movement

The calyx is short; the tube is about 1.5 mm long, the lowermost lobe about 2 mm long, the other lobes about 1 mm long. The standard extends a short distance forward at the base and then curves upward at a right angle or greater (Fig. 1). The flag part is 6–7 mm long and 6–8 mm wide. Two marked green-spots are found at the standard base (Fig. 2). The wing-keel complex is 6–8 mm long in the non-explored flower. The androecium is diadelphous, but the filaments make a column without any opening between the free stamen and the nine fusing stamens in the base (Figs. 5 and 6). The pistil in the column exposes only the distal part of the style, which curves weakly upward (Fig. 5). There are no nectaries in the flower.

Desmodium paniculatum blooms from late August to early October. The flowers open non-simultaneously in the morning (7–10 am). Intact flowers that have not been visited by insects extend the wing-keel complex at a right angle or greater to the standard (Fig. 3). The anthers and stigma are situated within the keel. After explosion, the wings and keel



Figs. 1–6. The flower of *Desmodium paniculatum*. 1: front view of the pre-explored flower. 2: the spots in the lower part of the standard. 3: side view of the pre-explored flower. 4: side view of the explored flower. 5: side view of the reproductive column comprising the diadelphous androecium and the pistil. 6: upside view of the basal part of the column, which shows no opening between one free stamen and nine fusing stamens. Bar = 2 mm.

curve down at a horizontal angle to the standard, but the column, consisting of the stamens and pistil, stays in the same place, resulting in exposure of the whole column (Fig. 4). The down ward curving movement of the wings and keel appear to be due to recurving in the basal part of the keel petals. Most pollen grains in the anthers, which have already dehisced, are spread during their enforced appearance through the adhering upper-edge of the keel, and some anthers may be pulled off from the filaments (Fig. 4). The stigmas were apparently receptive when they appeared. The flowers, even if not explored, began wilting around 3 pm.

Pollinators and their behavior

Honeybees (*Apis mellifera* L.) and solitary bees (*Nomia punctulata* Dalla Torre and *Megachile spissula* Cockerell) visited the flowers and were effective pollinators. The honeybees approached the flower frontally to touch the spots in the standard base with the antennae and landed on the wing-keel complex (Fig. 7). Body weight did not cause flower explosion, probably because the peduncles and also the branches with flowers were thin and flexible (Fig. 8). Explosion occurred just after the honeybees pushed their proboscis into the opening between the standard and the wings (Fig. 9). Flower explo-

Figs. 7–10. The bees of *Apis mellifera* visiting the flowers of *Desmodium paniculatum*. 7: the bee is landing on the wing-keel complex. 8: the bee inserts its proboscis into the opening under the spots in the standard. 9. the flower explored and the anthers and stigma touch the underside of the abdomen. 10: the bee is glooming while grasping the filament tube. Bar = 1 cm.

sion causes a forced appearance of the anthers from the keel inside to sprinkle the dry pollen grains onto the underside of bee abdomen, which touches the emergent anthers and stigma at the same time. Next, the bees usually grasped the stamens and sometimes also the keel and wings and groomed the body with the mid- and hind-legs to send pollen onto the pollen baskets. This process was completed in a short time, and the bees left the flower usually within a few seconds after landing on it. Honeybees rarely landed on the explored flowers even though they did approach them.

The solitary bees visited the flowers in almost the same way as the honeybees did, but they sometimes visited the explored flowers, although they soon left them without inserting proboscis into the flowers. The bees usually groomed in places other than on the flowers. Syrphid flies sometimes visited the explored flowers to lick the anthers, if presented, and the stigmas. Although the flies sometimes visited the non-explored flowers and appeared to look for something in the spotted area, they failed to obtain pollen.

Artificial explosion of the flower

Explosion of the flowers was artificially caused by insertion of a needle into the opening between the standard and the wings (Figs. 11, 12). The insertion appeared to bring about the explosion by the following process: the needle tip presses the column downwards in the inside of the opening and the downward movement of the column presses the basal part of the keel petals, which are tightly connected to the column. This pressure causes a strong recurvature of the keel base.

Anther number in the explored flowers

Anthers that remained in the flowers just after a bee-visit that led to an explosion, averaged only 1.2 in number (N = 33, SD = 1.8, range 0–7). Most anthers fell off by bee contact and a few ones pull off by the keel in the explosion. In the artificially explored flowers, 8.9 of the anthers were pulled off on average (N = 35, SD = 1.3, range 5–10).

UV absorption in the flower

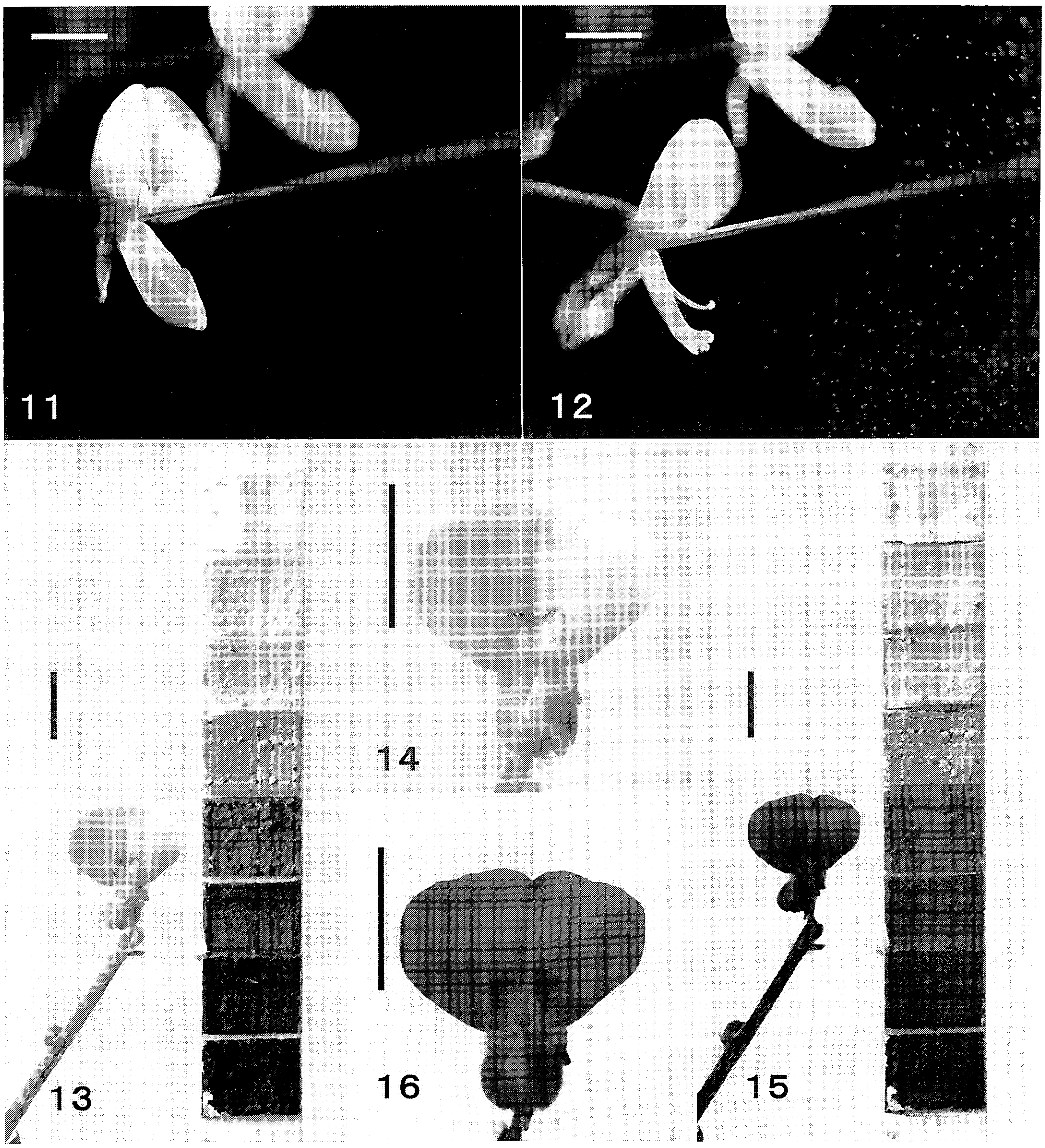
The spots on the standard base, each of which is composed of an upper light-green part and an under grayish-white part, completely absorbed UV light. The other area of the standard and wings, which are pink or bluish pink, also strongly absorbed (about 80 %) UV light (Figs. 13–16). These results show that the spots in the flower should be conspicuous for bees.

Glucose reaction in the flower

No glucose reactions were detected in every testable intact-surface of the flower; the calyx, corolla filaments and pistil, while glucose was clearly detected in almost all artificially injured parts in the concentration of about 1 mg/ml. It was impossible to intactly examine the ovary base because the filament tube tightly covered it.

Discussion

Explosion in the *Desmodium paniculatum* flower is elegant. In the papilionate flowers of Leguminosae, some outside fource by pollinators is needed to expose the stamens or pollen and the stigma, which are concealed in the keel (Faegri and van der Pijl 1971). The most important force is the downward press from the legs of pollinators, which is principally the same in the explosive flowers (Faegri and van der Pijl 1971, Westercamp 1997, Galloni and Cristofolini 2003) although the stamens and style do not come back into the keel. In *D. paniculatum*, however, a needle-insertion into the opening between the standard- and the wing-base easily causes explosion; showing that bee-proboscis insertion is enough to explode the flower without any other force. The explosion occurs almost simultaneously with in-



Figs. 11–16. Artificial explosion with a needle in the flower of *Desmodium paniculatum* (Figs. 11, 12), and UV absorption of the flower (Figs. 13–16). 11: a needle is inserted into the opening between the standard- and the wing- base. 12: the flower is explored and a cloud of pollen is spread. 13 and 14: photographs under visual light. 15 and 16: photographs under UV light. Bar = 0.5 mm.

sertion.

Desmodium paniculatum possesses many of the features that have been described in explosive flowers; no return to original position in the wings and keel, spread of pollen cloud at flower explosion, rare re-visit of pollinators, and lack of nectar. The anthers fall out easily, and only a few anthers were

left on the filaments after one bee-visit. Pollen seems to be the only reward for pollinators. Although it had been generally believed that explosive flowers produced no or little nectar (Kugler 1955, Faegri and van der Pijl 1971), *Medicago sativa* (Free 1970) and *M. citrine* (Perez-Banon et al. 2003) secrete much nectar. Galloni and Crisatofolini (2003) showed that *Spartium junceum*, *Genista cilentina*, and *G. radiata* produced nectar at the base of the standard or on the reproductive column. They observed some nectar-feeding insects, such as long-tongued Anthophora, and male solitary bees on their flowers. Insertion of bee-proboscis into the opening between the standard and the wings in the *Desmodium paniculatum* flower is apparently similar to the nectar-seeking behavior in many legume species. The bees, however, did not appear to target nectar, because they quickly pulled their proboscis out and left the flowers in a short time, which was enough for taking pollen. Nevertheless, the bees, which tend to fly out from rewardless populations, continued to visit many flowers in the same population of *D. paniculatum*.

The marked spots in the standard of the *D. paniculatum* flower are very similar to the nectar guides that are common in nectariferous flowers of Papilionoideae, although many of them are lines that converge toward the entrance to the flower (Kevan 1983). In *D. panuculatum*, however, the spots appear to function as a guide mark leading to flower explosion. The bees always inserted their proboscis into the opening under the spots on the nectarless flowers. Barth (1985) showed some examples of pollen guides that introduce visitors to the pollen location in anther-hidden flowers. Most of them are imitations of anthers or pollen, contrasting to nectar guides in which their designs appear to be unrelated to any food source. The spots of *Desmodium paniculatum* are quite unique because they are very similar to a nectar guide and do not directly indicate pollen-existing places. They may be called an "explosion guide." It is hypothesized that the explosion guide is homologous with a "true" nectar guide, and that *D. paniculatum* was derived from nectariferous species because it has diadelphous stamens, which are usually found in nectariferous species of Papilionoideae. Nonetheless, the pollinators behave as if they know well how to obtain the pollen. The pollen-collecting bees may learn how to obtain pollen first by the experience of inserting their proboscis into the tube to seek nectar.

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高橋　弘：アレチヌスビトハギの爆裂花における蜜標類似マークの役割

アレチヌスビトハギは北アメリカ原産で，日本の広い地域に帰化している．この花は典型的な“爆裂花”で，一度爆裂すると翼弁と舟弁は下方へ降りたまま元に戻らず，爆裂時に花粉を雲状にまき散らし，爆裂後は送粉者であるハナバチはほとんど訪れず，また花蜜を出さない．爆裂はハナバチによる翼弁—舟弁複合体の押し下げによるのではなく，口吻を旗弁と翼弁の基部の隙間に挿入することにより引き起こされる．他の力を要しないことは，針の挿入だけで爆裂させ得ることにより明らかである．旗弁の基部にはマメ亜科の多くの種に見られる蜜標に似た斑紋がある．しかし，アレチヌスビトハギは蜜を分泌しないので「蜜標」とは言えない．訪れたハナバチは，吸蜜を目的としてこの標識の下の隙間に口吻を挿入しているようには見えない．ハナバチ，特にミツバチは，花蜜を求めて訪花する場合，報酬物が少ない集団でいつまでも探査を続けることはない．ハチは，口吻を挿入して花が爆裂すると，直ちに口吻を引き抜いて花粉収集に熱中する．すなわち，標識の下の隙間に口吻を挿入すると花が爆裂して花粉が得られることを知っているかのように振る舞うのである．しかし，アレチヌスビトハギは2体雄蕊を持つことから，花蜜を生産する群から進化した可能性が高く，爆裂誘導指標は蜜標から由来したものと考えられる．

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